Application Note

Automatic pipetting of reaction mixtures for PCR in 8-well strips and qPCR in 384-well format

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Introduction

PCR and quantitative Real Time PCR (RT-qPCR) are standard techniques in the field of molecular biology. In both methods, specific segments of DNA or transcribed RNA are amplified and analyzed. In standard PCR, qualitative analysis of amplified products is carried out with the help of gel electrophoresis. During RT-qPCR, the amount of amplified DNA is detected in real time by using intercalating dyes. PCR and RT-qPCR are used in a variety of applications in molecular biology; for example, for genotyping transgenic mice and for gene expression analyses of cell cultures.

Material and methods

Genotyping

For genotyping, the tissue of the transgenic mice (ear or tail biopsy) is first lysed and then the DNA is isolated via isopropanol isolation. A PCR and subsequent gel electrophoresis procedure are carried out to detect transgenic animals or knockouts. Pipetting the PCR reaction mixture, consisting of master mix, primer, water and sample, is done using the Liquid Handling Station (LHS).

Using the Liquid Handling Station to pipette PCR mixtures for genotyping

The complete reaction mixture of a PCR procedure is pipetted for 44 samples and one non-template control (NTC) using the Liquid Handling Station. First, the corresponding master mix volumes are pipetted. After mixing, the volume for a sample quantity is dispensed into 8-well PCR strips, and then the sample is pipetted into the reaction mixture.

Table 1: Pipetting method for one PCR mixture for 44 samples and 1 NTC.

Demand	Liquid End	Transfer
Transfer 3 x192 µL dream mix to master mix	200 µL	Rack1 Tube D3 → D6
Transfer 92 µL primer to master mix	200 µL	Rack1 Tube D4 → D6
Transfer 23 µL H_20 to master mix	200 µL	Rack1 Tube D5 → D6
Mix master mix	200 µL	
Transfer 15 µL master mix to PCR mix	200 µL Multidispense	Rack 1 Tube D6 → 8-Strips A1-A8, B1-B8, C1-C8, D1-D8, E1-E8, F1-F4
Transfer 10 µL sample into PCR mix	50 µL	Rack 1 Tube A1-D2, Rack 2 Tube A1-D6 → 8- Strips A1-A8, B1-B8, C1-C8, D1-D8, E1-E8, F1-F4

Gene expression analyses with RT-qPCR in 384-well format

For the quantitative Real Time PCR procedure, first the isolation and purification of RNA from cell cultures takes place. Then RNA is transcribed into cDNA. The qPCR procedure is carried out in triplicate in 384-well plates.

Use of the Liquid Handling Station for pipetting RT-qPCR mixtures into a 384-well format

8 different PCR mixtures, comprised of SybrGreen master mix, Rox, UNG, primer, and water are provided in tubes, according to the sample quantities. Using the Liquid Handling Station, master mixes are transferred to a 96-well plate; in addition, 4 samples and one NTC each are pipetted. Following this, triplicates are pipetted into a 384-well plate from the 96-well plate with the 8-channel Liquid End.

Table 2: Pipetting method for 8 RT-qPCR mixtures for 4 samples and 1 NTC.

Demand	Liquid End	Transfer
Transfer 56 µL master mix to 96-well plate for each mixture	200 µL	Rack1 Tube A1 → Rack 96-Well A1-5, B1-5, C1-5, D1-5, E1-5, F1-5, G1-5, H1-5
Transfer 14 µL sample to 96-well plate for each mixture	50 µL	Rack1 Tube A5, B5, C5, D5, A6 → Rack 96 Well A1-5, B1-5, C1-5, D1-5, E1-5, F1-5, G1-5, H1-5
Transfer 3 x 20 µL qPCR mix to 384-well plate	50µL 8 channel	Example for a sample of 8 reaction mixtures: Rack 96-Well A1, B1, C1, D1, E1, F1, G1, H1 → Rack 384 –Well A1-3, C1-3, E1-3, G1-3, I1-3, K1-3, M1-3, O1-3

Results

The Liquid Handling Station enables automated and reliable pipetting of PCR mixtures for up to 44 samples for genotyping. When handling large sample quantities, the Liquid Handling Station has a clear advantage over manual pipetting, as it consistently operates error-free with the proper programming. The sample quantities can be increased through additional adapters.

The Liquid Handling Station is indispensable for pipetting RT-qPCR mixtures into a 384-well format. The pipetting accuracy is comparable with a manual pipette. This can be seen in Figure 1, which shows the amplification of different genes of triplicates. Pipetting errors, which can certainly happen when manually pipetting in 384-well format, are eliminated by using the Liquid Handling Station.

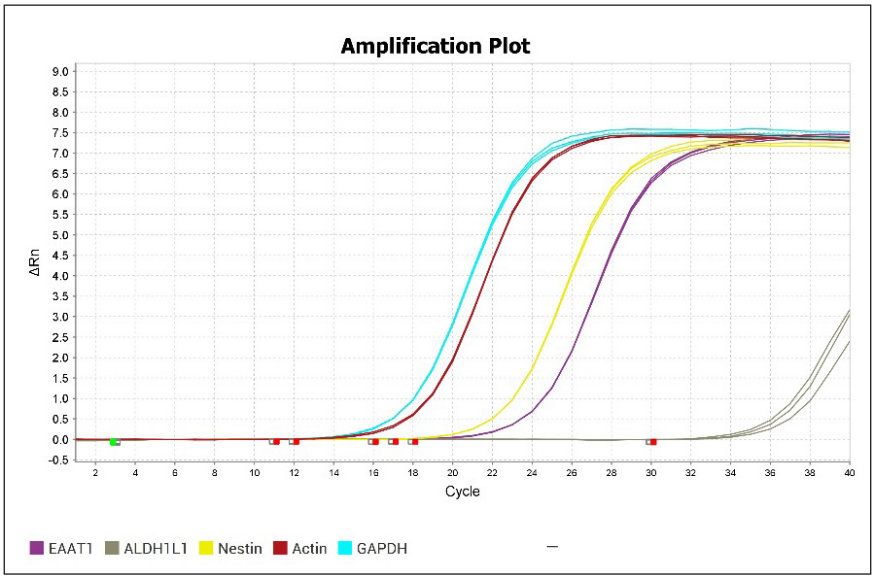

Figure 1: Amplification plots of triplicates of a RT-qPCR

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